

Determining Possible Fragments Based On Experimental Restrictions

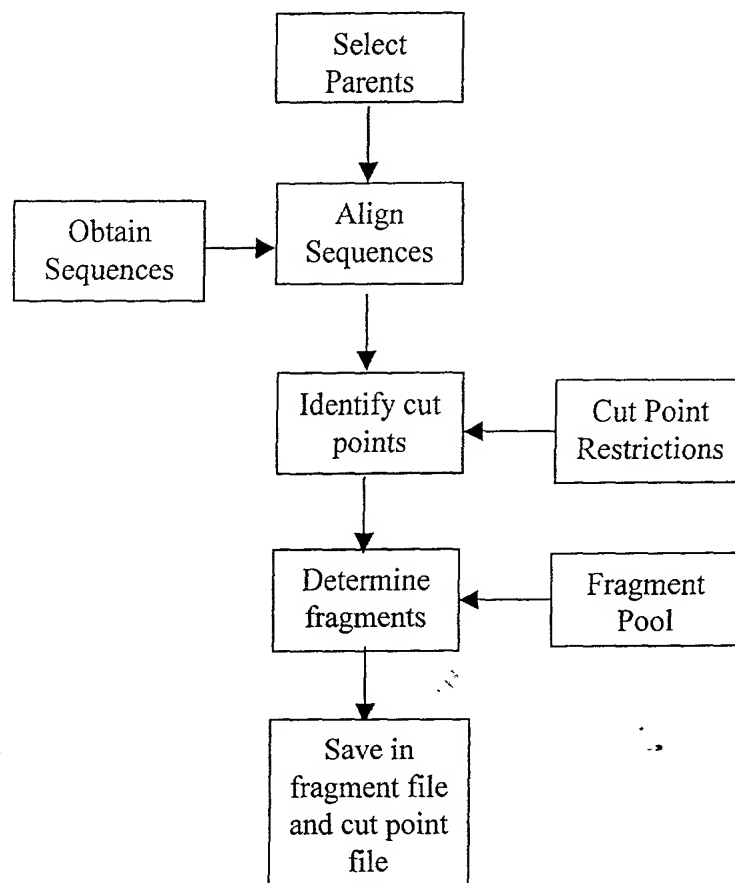


FIG. 1A

Determining the Schema Disruption Profile for a Structure

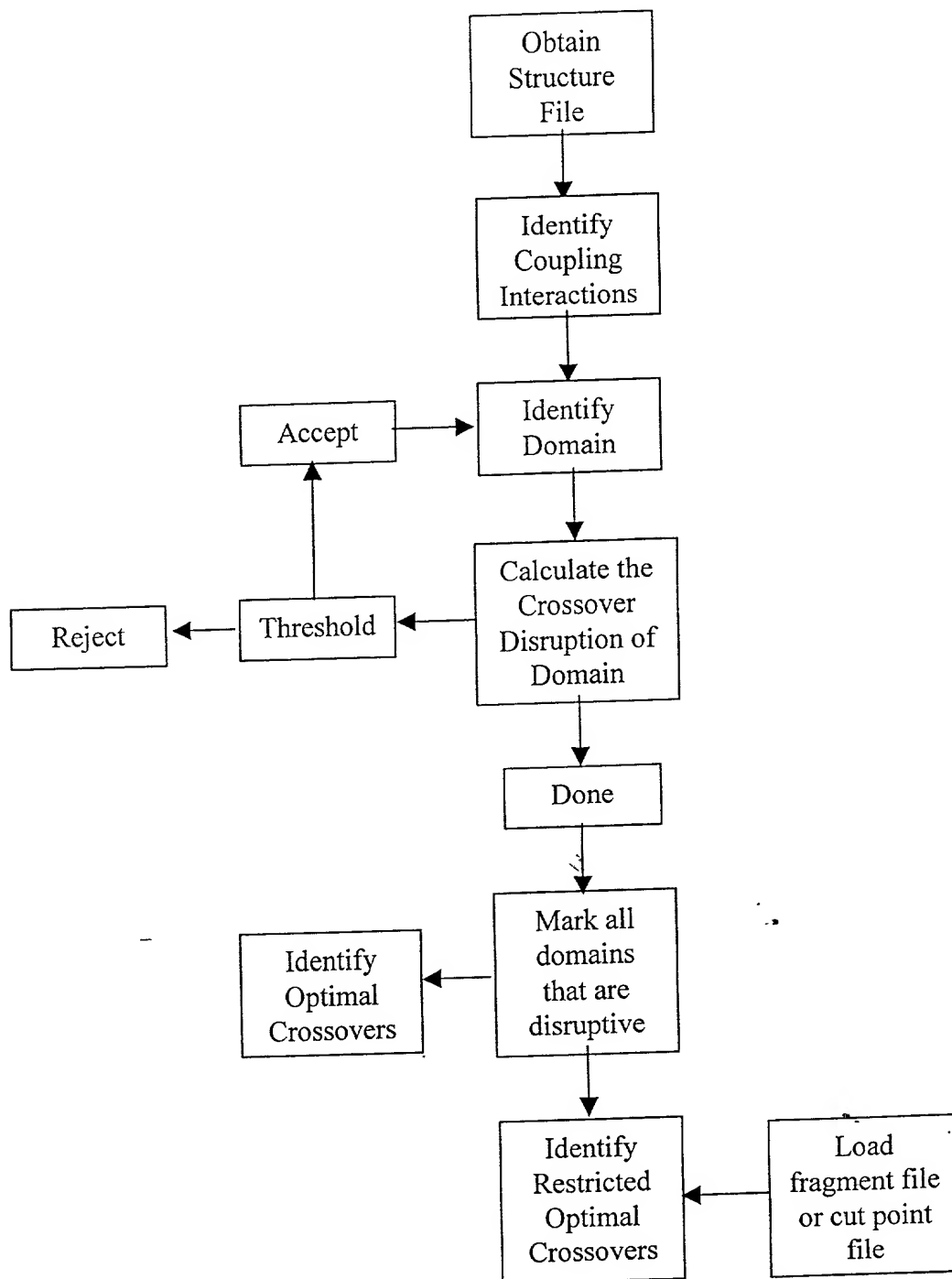


FIG. 1B

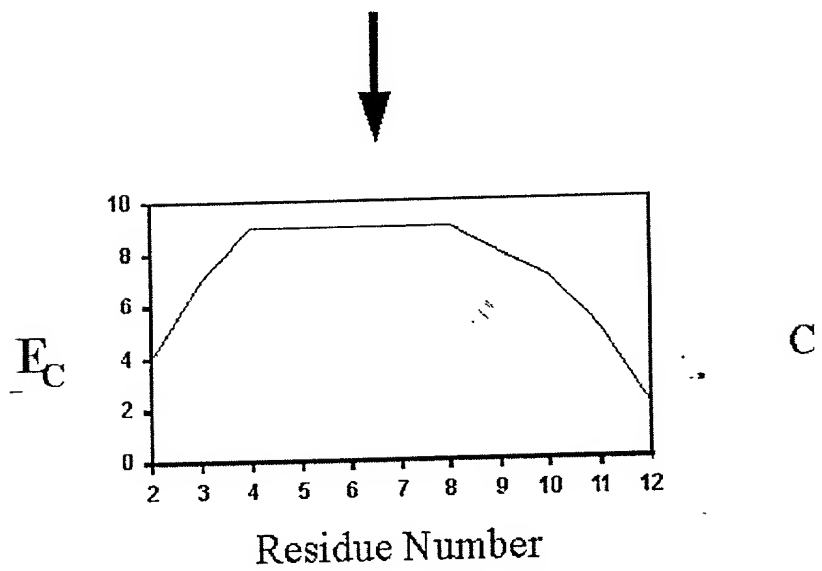
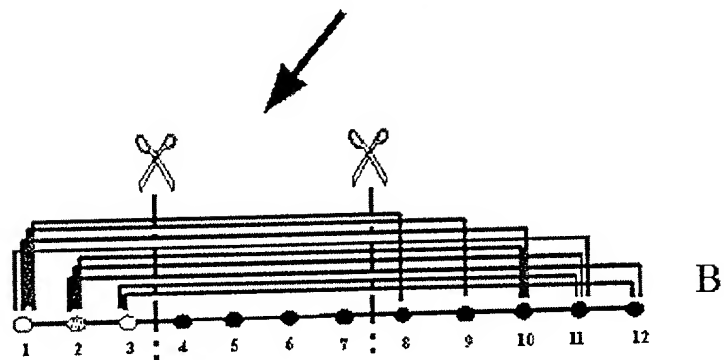
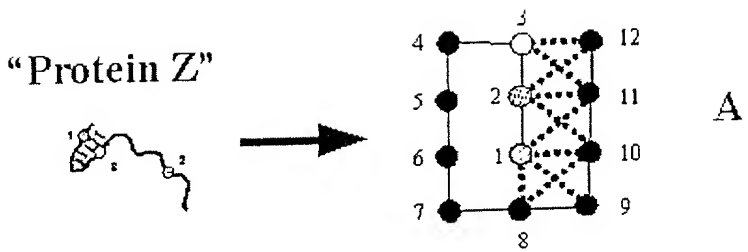


FIG. 2

1	1	T	P	V	S	E	E	K	Q	L	A	E	V	A	N	T	I	T	P	L	M	K	A	Q	S	V	P	G	M	A	V	A	V	I	Y	Q	G	K	P	P	Y	Y	T	F	G	K	A	D	I	A	A	
2	1	A	K	L	T	E	L	Q	Q	I	A	D	I	V	N	N	T	I	T	P	L	M	K	A	Q	S	V	P	G	M	A	V	A	V	I	Y	Q	G	K	P	P	Y	Y	T	F	G	K	A	D	I	A	A
3	1	T	K	L	T	E	L	Q	Q	I	A	D	I	V	N	N	T	I	T	P	L	M	K	A	Q	S	V	P	G	M	A	V	A	V	I	Y	Q	G	K	P	P	Y	Y	T	F	G	K	A	D	I	A	A
4	1	Y	A	R	G	E	A	P	L	T	A	A	V	A	N	N	T	I	T	P	L	M	K	A	Q	S	V	P	G	M	A	V	A	V	I	Y	Q	G	K	P	P	Y	Y	T	F	G	K	A	D	I	A	A
1	51	N	K	P	V	T	P	Q	T	L	F	E	L	G	S	I	S	K	T	F	T	G	V	L	G	G	D	A	I	A	R	G	E	I	S	L	D	D	A	V	T	R	Y	W	P	Q	L	T	G	K	Q	
2	51	N	H	R	P	V	T	P	Q	T	L	F	E	L	G	S	I	S	K	T	F	T	G	V	L	G	G	D	A	I	A	R	G	E	I	S	L	D	D	A	V	T	R	Y	W	P	Q	L	T	G	K	Q
3	51	G	Q	R	P	V	T	E	N	T	L	F	E	L	G	S	I	S	K	T	F	T	G	V	L	G	G	D	A	I	A	R	G	E	I	S	L	D	D	A	V	T	R	Y	W	P	Q	L	T	G	K	Q
4	51	G	Q	R	P	V	T	E	N	T	L	F	E	L	G	S	I	S	K	T	F	T	G	V	L	G	G	D	A	I	A	R	G	E	I	S	L	D	D	A	V	T	R	Y	W	P	Q	L	T	G	K	Q
1	101	W	Q	G	I	R	M	L	L	D	L	A	T	Y	T	A	G	G	L	P	L	Q	V	P	D	E	V	T	D	N	A	S	L	L	R	F	Y	Q	N	W	Q	P	Q	W	T	P	G	A	K	R	L	Y
2	101	W	R	G	I	R	M	L	L	D	L	A	T	Y	T	A	G	G	L	P	L	Q	V	P	D	E	V	T	D	N	A	S	L	L	R	F	Y	Q	N	W	Q	P	Q	W	T	P	G	A	K	R	L	Y
3	101	W	K	D	V	K	M	L	L	D	L	A	T	Y	T	A	G	G	L	P	L	Q	V	P	D	E	V	T	D	N	A	S	L	L	R	F	Y	Q	N	W	Q	P	Q	W	T	P	G	A	K	R	L	Y
4	101	F	D	G	V	T	M	A	E	L	A	T	Y	T	A	G	G	L	P	L	Q	V	P	D	E	V	T	D	N	A	S	L	L	R	F	Y	Q	N	W	Q	P	Q	W	T	P	G	A	K	R	L	Y	
1	151	A	N	S	I	G	L	F	G	A	L	A	V	K	P	S	G	M	P	Y	E	Q	A	M	T	T	R	V	L	K	P	L	K	L	D	H	T	W	I	N	V	P	K	A	E	E	A	H	Y	A		
2	151	A	N	S	I	G	L	F	G	A	L	A	V	K	P	S	G	M	P	Y	E	Q	A	M	T	T	R	V	L	K	P	L	K	L	D	H	T	W	I	N	V	P	K	A	E	E	A	H	Y	A		
3	151	S	N	A	S	I	G	L	F	G	A	L	A	V	K	P	S	G	M	P	Y	E	Q	A	M	T	T	R	V	L	K	P	L	K	L	D	H	T	W	I	N	V	P	K	A	E	E	A	H	Y	A	
4	151	S	N	A	S	I	G	L	F	G	A	L	A	V	K	P	S	G	M	P	Y	E	Q	A	M	T	T	R	V	L	K	P	L	K	L	D	H	T	W	I	N	V	P	K	A	E	E	A	H	Y	A	
1	201	W	G	Y	R	D	G	K	A	V	R	V	S	P	G	M	L	D	A	Q	A	Y	G	V	K	T	N	V	Q	D	M	A	N	W	V	M	A	N	M	A	P	E	N	V	A	D	A	S	L	K	Q	
2	201	W	G	Y	L	E	G	K	P	V	R	V	S	P	G	M	L	D	A	Q	A	Y	G	V	K	T	N	V	Q	D	M	A	N	W	V	M	A	N	M	A	P	E	N	V	A	D	A	S	L	K	Q	
3	201	W	G	Y	L	E	G	K	P	V	R	V	S	P	G	M	L	D	A	Q	A	Y	G	V	K	T	N	V	Q	D	M	A	N	W	V	M	A	N	M	A	P	E	N	V	A	D	A	S	L	K	Q	
4	201	Y	G	Y	K	E	D	K	P	V	R	V	S	P	G	M	L	D	A	Q	A	Y	G	V	K	T	N	V	Q	D	M	A	N	W	V	M	A	N	M	A	P	E	N	V	A	D	A	S	L	K	Q	
1	251	G	I	A	L	A	Q	S	R	Y	W	R	I	G	S	M	Y	Q	G	L	G	W	E	M	L	N	W	P	V	E	A	N	T	V	V	E	G	S	D	S	K	V	A	L	A	P	L	P	A	E		
2	251	G	I	A	L	A	Q	S	R	Y	W	R	I	G	S	M	Y	Q	G	L	G	W	E	M	L	N	W	P	V	E	A	N	T	V	V	E	G	S	D	S	K	V	A	L	A	P	L	P	A	E		
3	251	R	I	A	L	A	Q	S	R	Y	W	R	I	G	S	M	Y	Q	G	L	G	W	E	M	L	N	W	P	V	E	A	N	T	V	V	E	G	S	D	S	K	V	A	L	A	P	L	P	A	E		
4	249	R	I	A	L	A	Q	S	R	Y	W	R	I	G	S	M	Y	Q	G	L	G	W	E	M	L	N	W	P	V	E	A	N	T	V	V	E	G	S	D	S	K	V	A	L	A	P	L	P	A	E		
1	301	V	N	P	A	P	V	K	A	S	W	V	H	K	T	G	S	T	G	G	F	G	S	Y	V	A	F	I	P	E	K	Q	I	G	I	V	M	L	A	N	K	S	Y	P	N	P	A	R	V			
2	301	V	N	P	A	P	V	K	A	S	W	V	H	K	T	G	S	T	G	G	F	G	S	Y	V	A	F	I	P	E	K	Q	I	G	I	V	M	L	A	N	K	S	Y	P	N	P	A	R	V			
3	301	L	V	P	A	P	V	K	A	S	W	V	H	K	T	G	S	T	G	G	F	G	S	Y	V	A	F	I	P	E	K	Q	I	G	I	V	M	L	A	N	K	S	Y	P	N	P	A	R	V			
4	299	F	A	V	P	K	A	M	G	E	Q	R	L	Y	N	K	T	G	S	T	G	G	F	G	A	Y	V	A	F	I	P	E	K	Q	I	G	I	V	M	L	A	N	K	S	Y	P	N	P	A	R	V	
1	351	E	A	A	Y	H	I	L	E	A	L	Q																																								
2	351	K	A	A	W	R	I	L	E	K	L	Q																																								
3	351	Q	A	A	Y	D	I	L	Q	A	L	R																																								
4	349	K	A	A	Y	D	I	L	Q	A	L	R																																								

FIG. 3

- | | | |
|---|-------------------------|-----------------|
| 1 | Enterbacter cloacae | P05364 (X03866) |
| 2 | Citrobacter freundii | P05193 (X07274) |
| 3 | Yersinia enterocolitica | P45460 (X63149) |
| 4 | Klebsiella pneumoniae | Q48437 (X77455) |

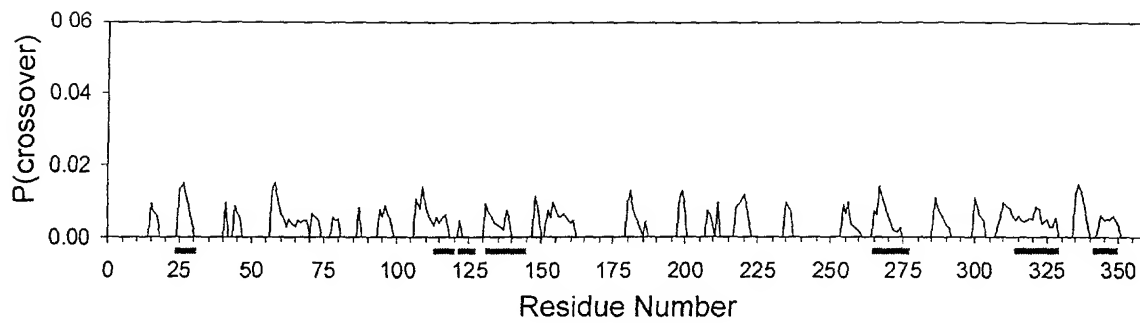


FIG. 4A

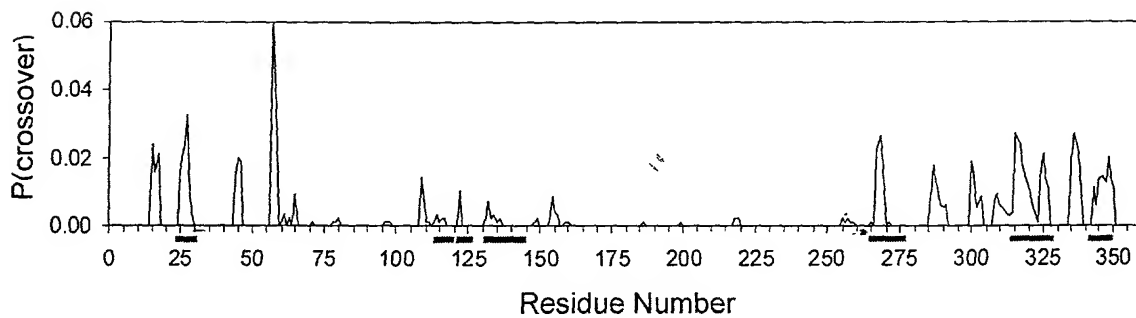


FIG. 4B

40016668-103604

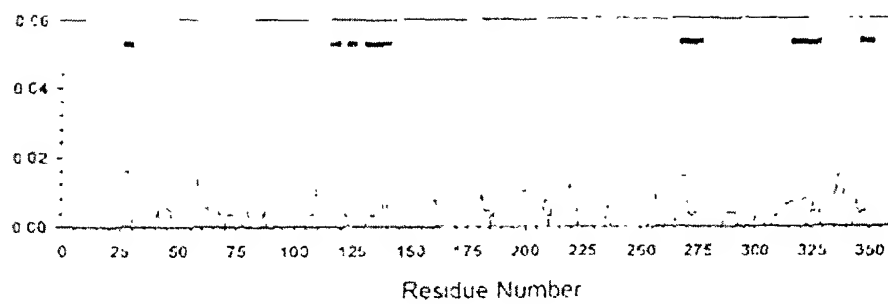


FIG. 4C

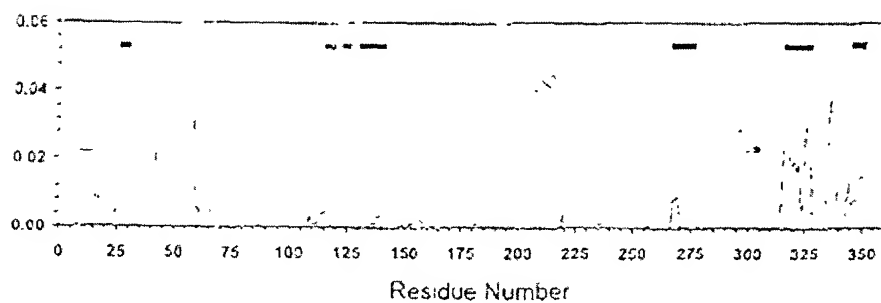


FIG. 4D

10016668-10301
FOOT-8999FOOT

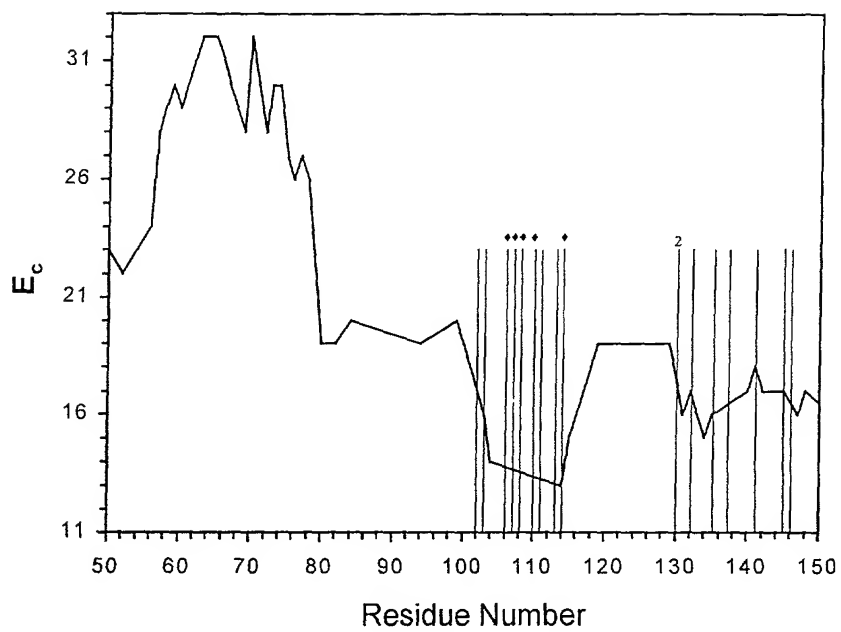


FIG. 5

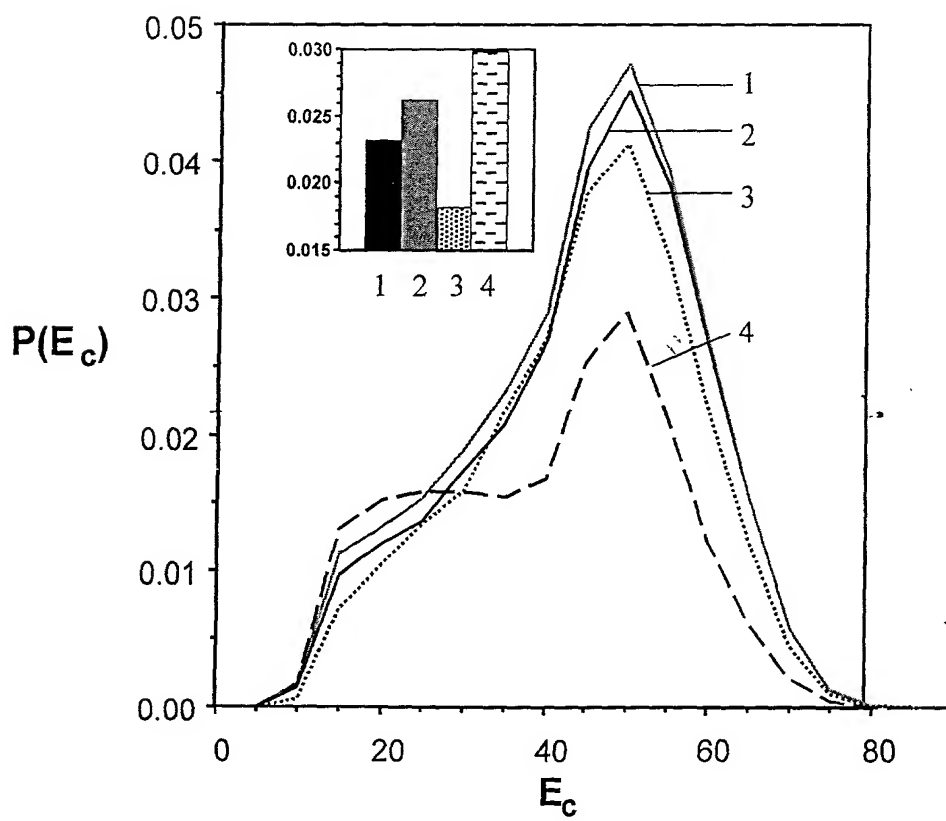


FIG. 6

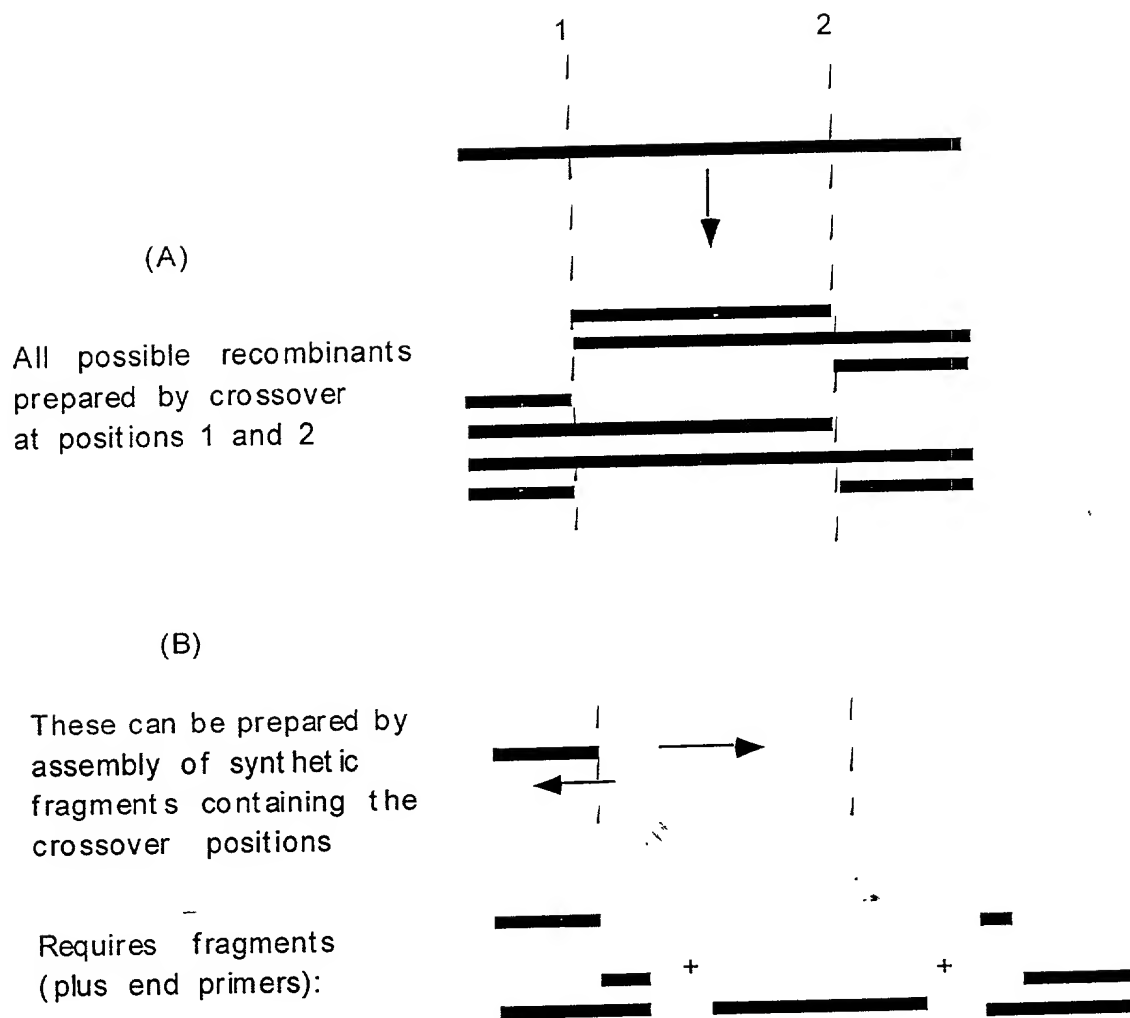


FIG. 7

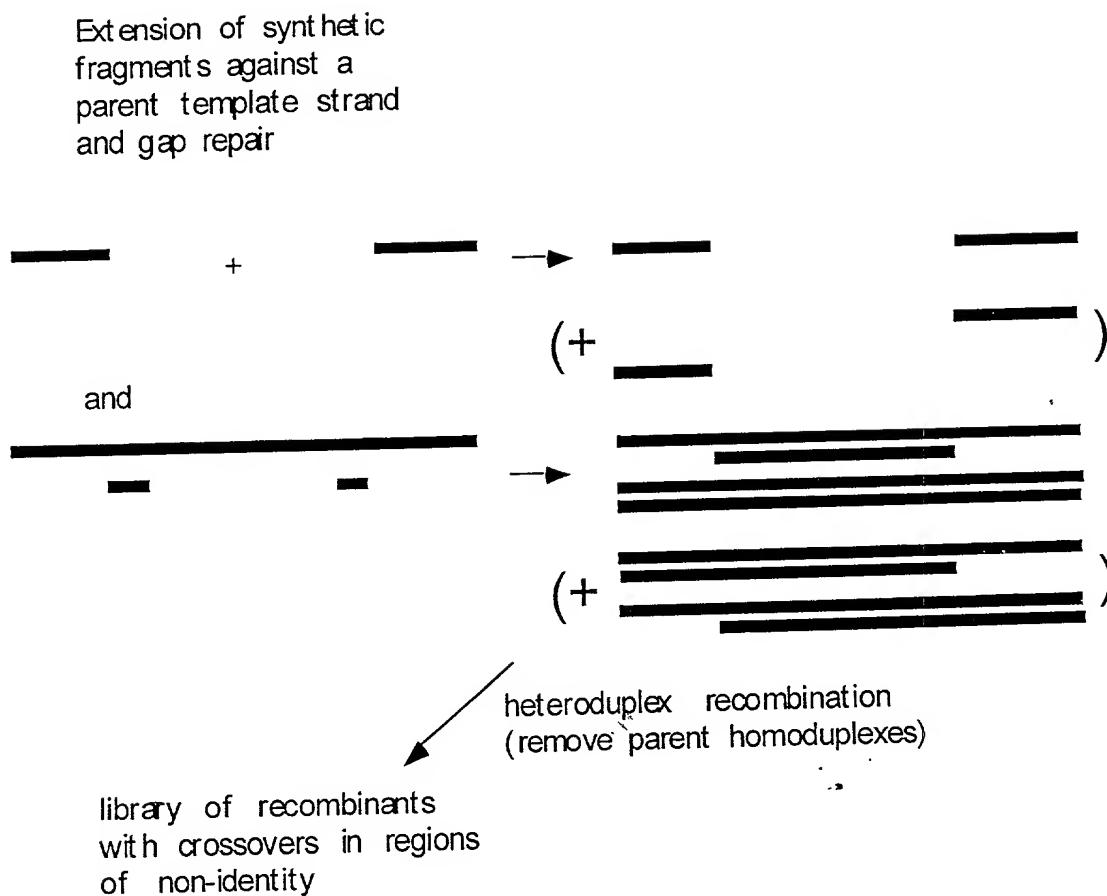
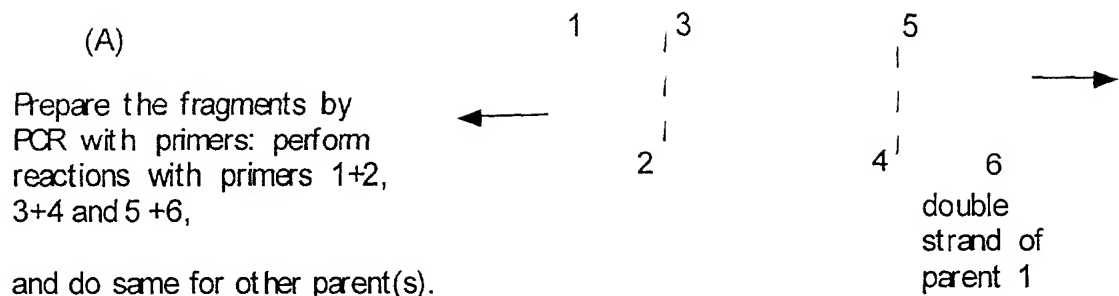


FIG. 8

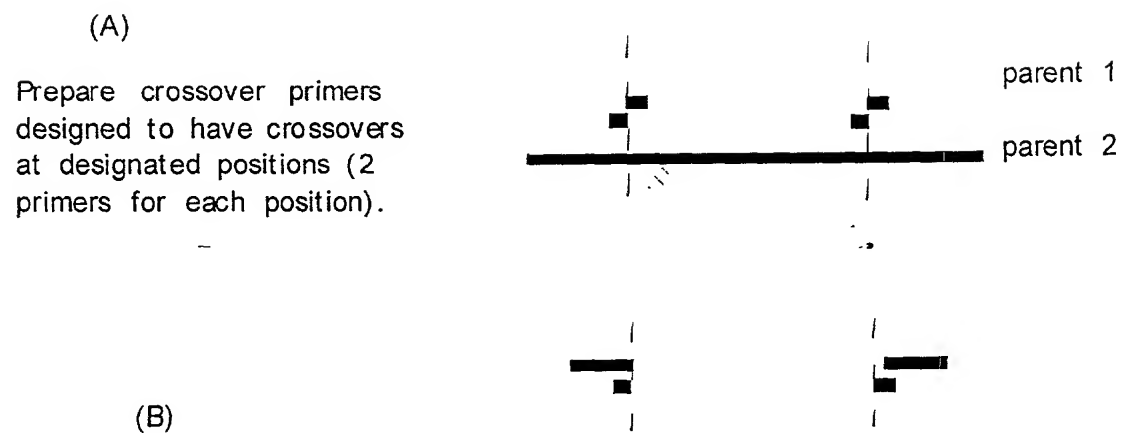
10016553 102601



(B)

Reassemble fragments in a pool, by PCR with 1+ 6

FIG. 9



Fragment parent genes and PCR reassemble in the presence of the crossover primers to promote recombination at designated positions

FIG. 10

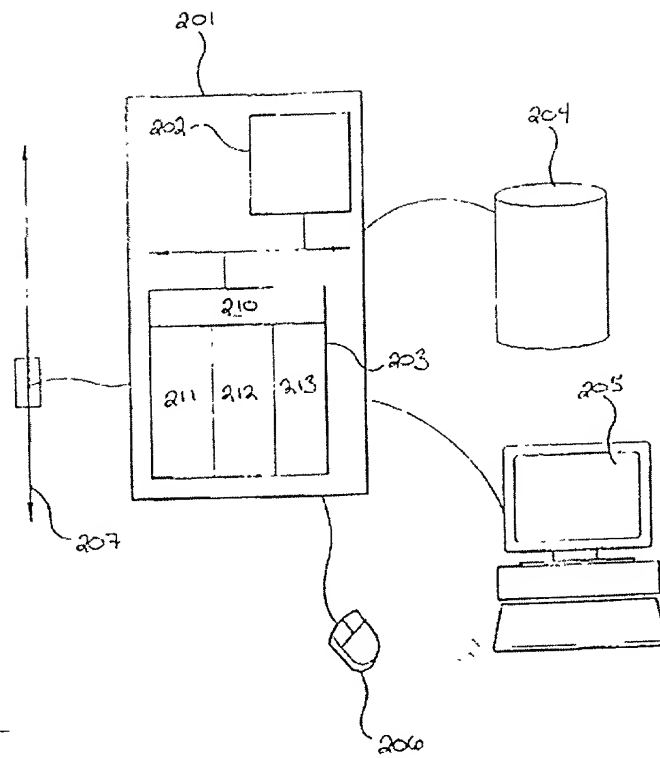
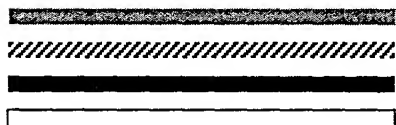


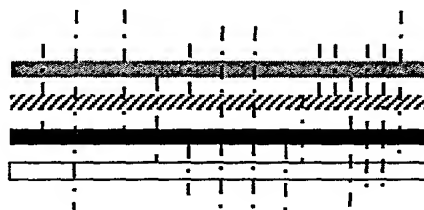
FIG. 11

Recombinant search algorithm

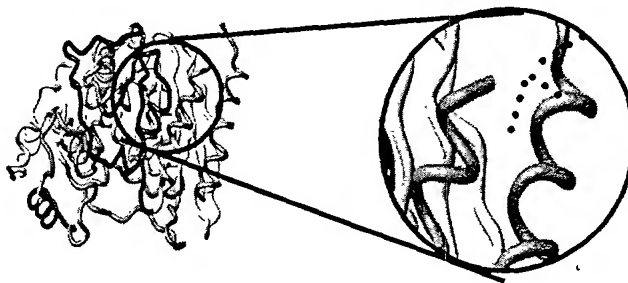
1. Align parent sequences
with template structure



2. Determine all possible crossover points
according to sequence identity algorithm



3. Calculate coupling matrix



4. Pick start parent at random
and copy to offspring until a
possible cut point is reached

5. Pick random number, if less than p ,
copy random new parent until next cut
point is reached.

6. Determine crossover
disruption of offspring gene

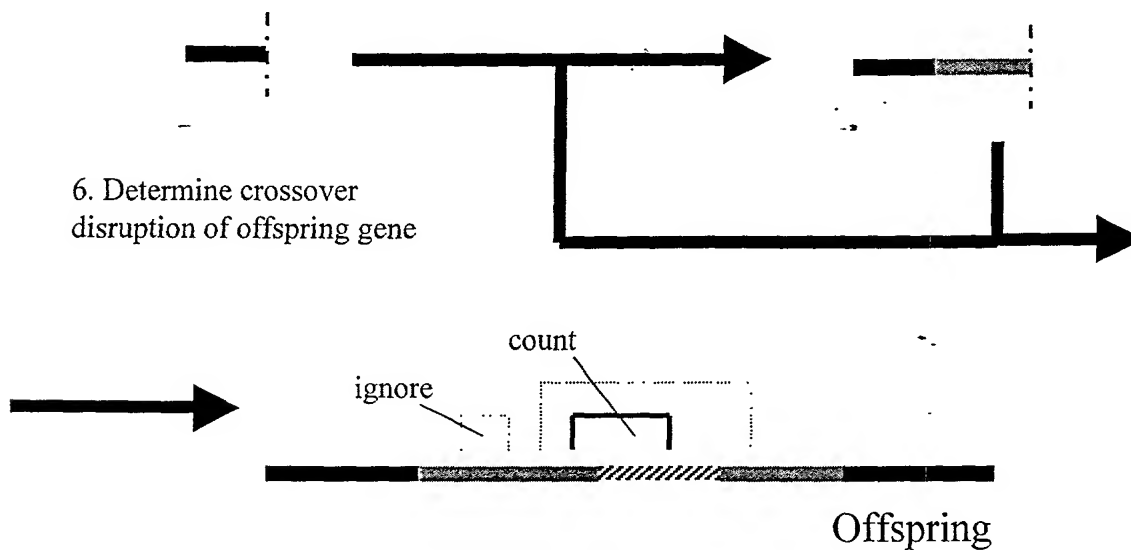


FIG. 12

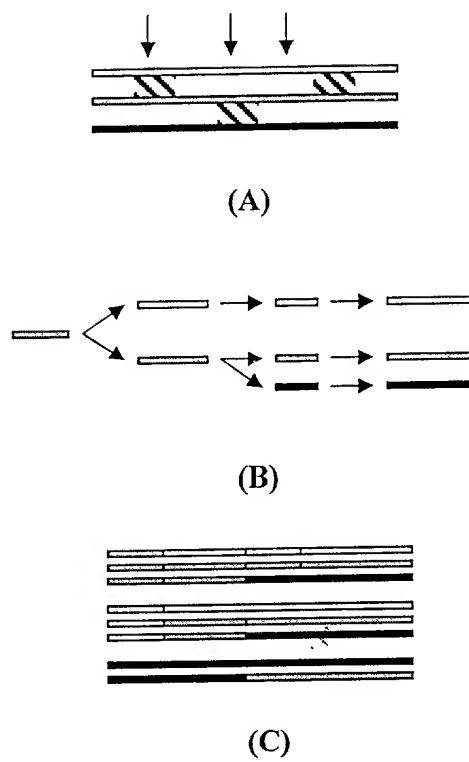


FIG. 13

DIRECTED EVOLUTION ALGORITHM

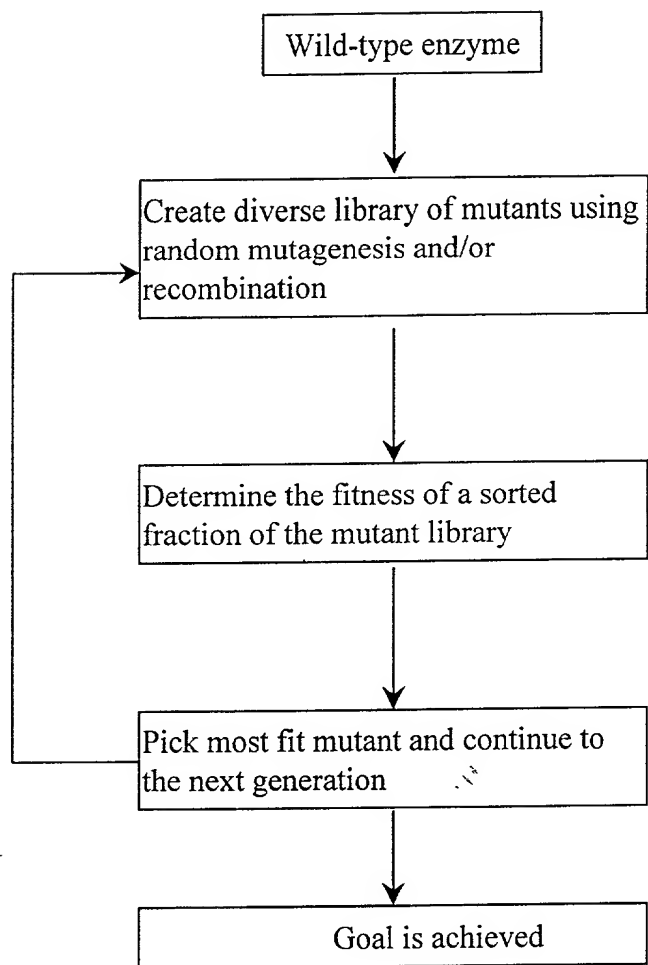


FIG. 14

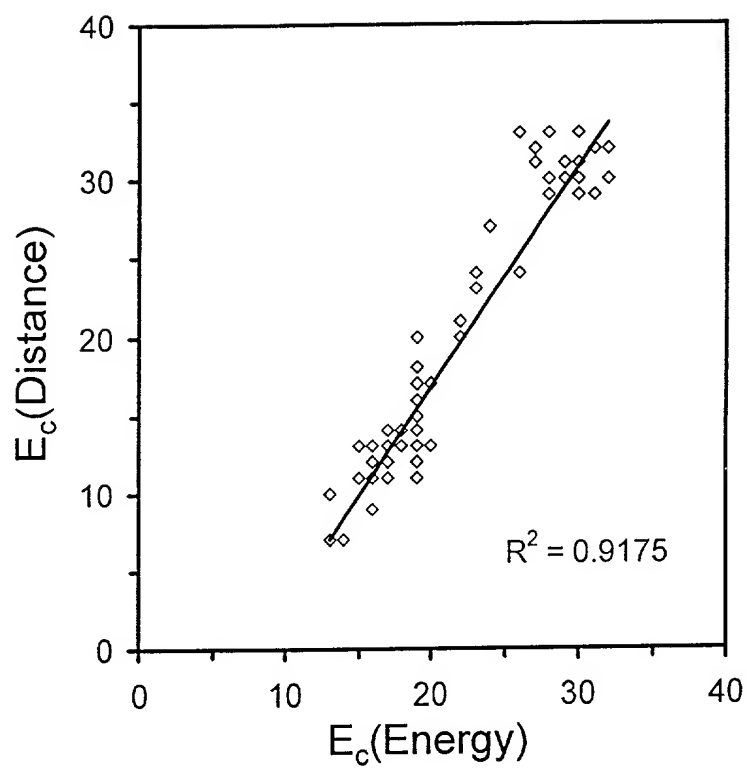


FIG. 15

10016668-102601

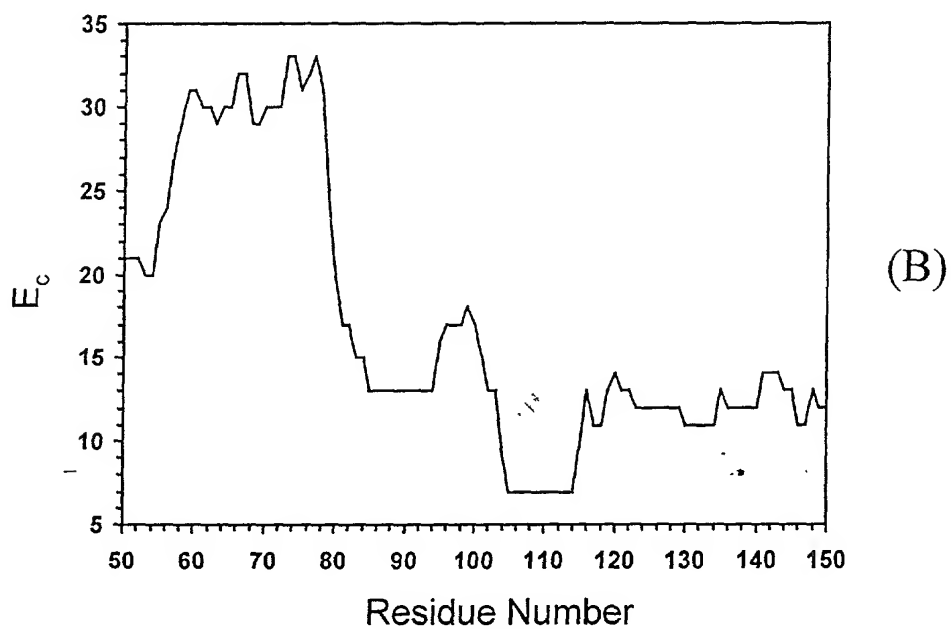
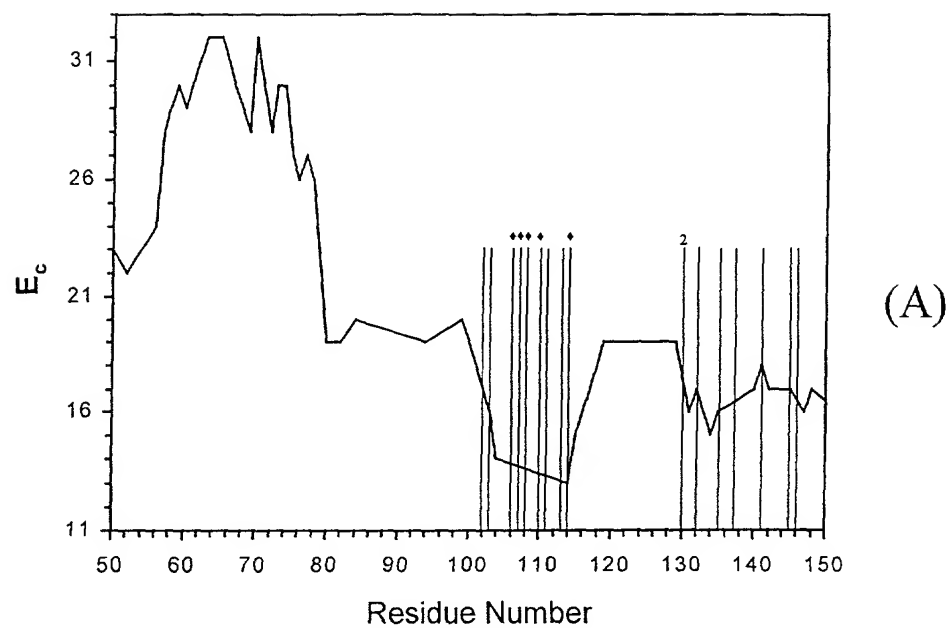


FIG. 16



(A)

Experimental Data:

	wt	wt-insert	1	2
Tm(dC)	52	55.2	n.d.	54.3
Tm(dC)	49.5	53.3	44.5	52.5
t1/2	12.1	2586	-	87.5
t1/2	53	138	4	308

(B)

Calculations:

	All schema		Fragments		Z-score	
	av	stdev	1	2	1	2
Ec	19.260	4.090	10.770	8.124	-2.076	-2.723
Ec*	0.006	0.002	0.014	0.005	4.838	-0.857

FIG. 17

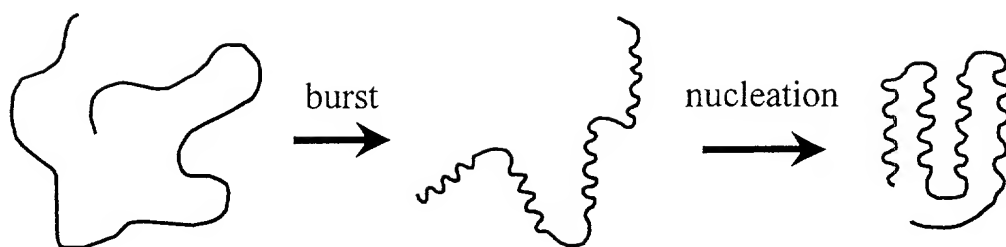



FIG. 18

The contact map shows residues that are distant (black) and residues that are close (white). If a given segment, , folds an above average number of residues into a given sphere size, then it is compact.

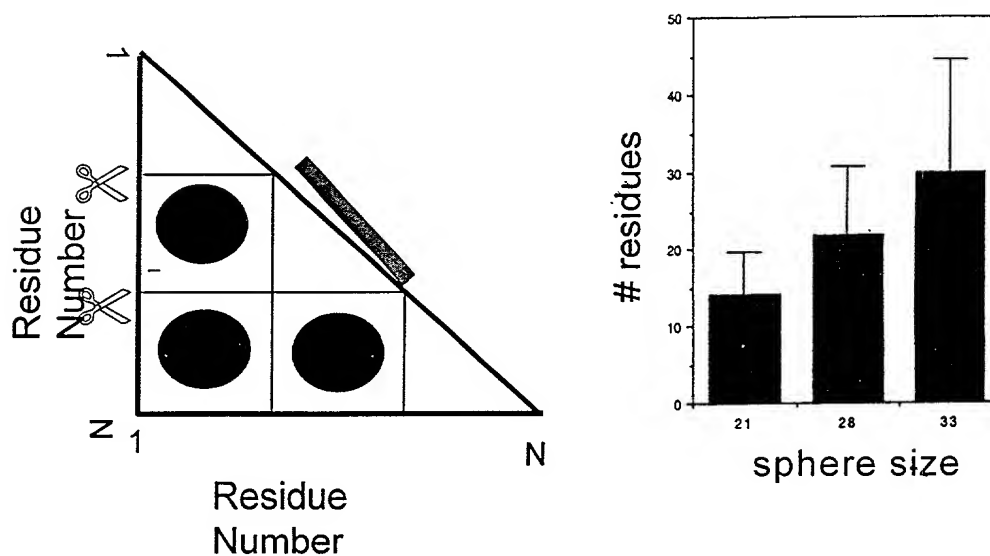


FIG. 19

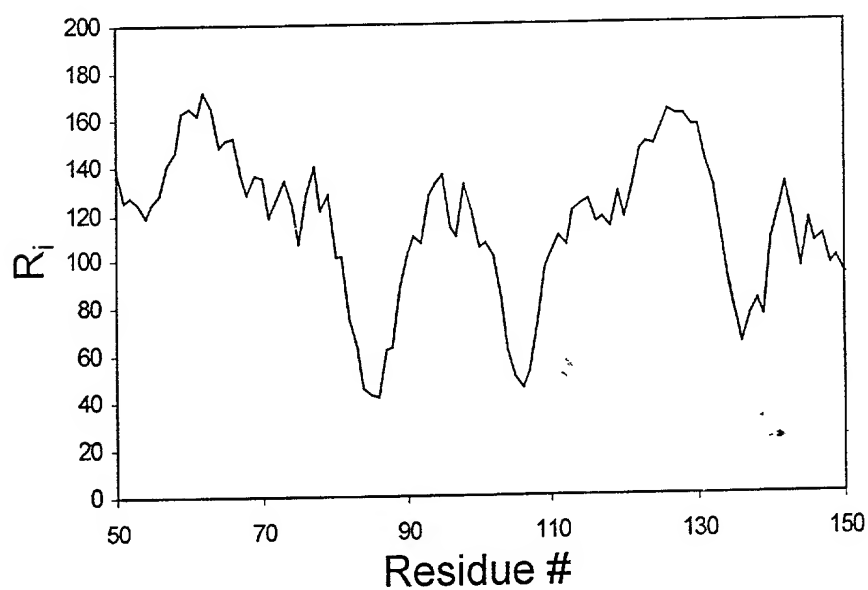
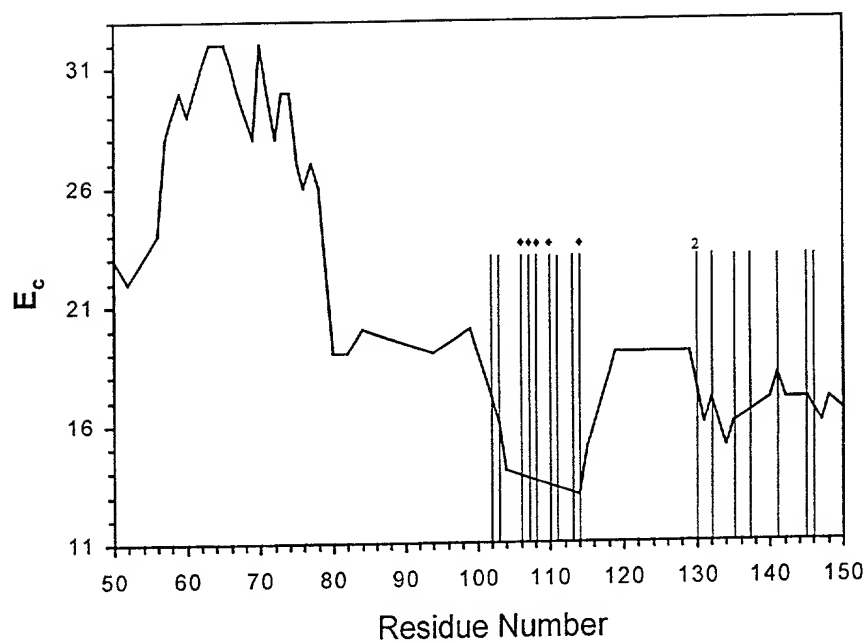


FIG. 20

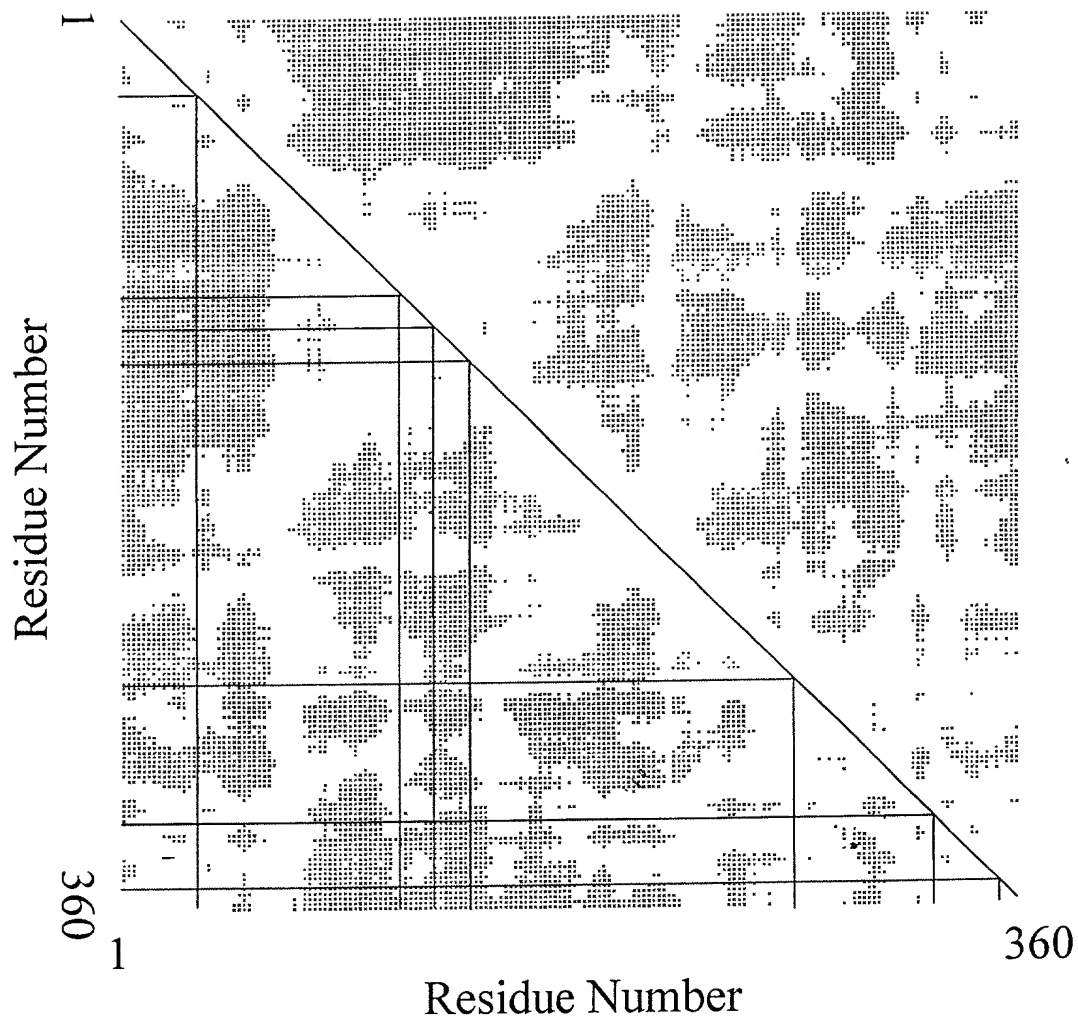


FIG. 21

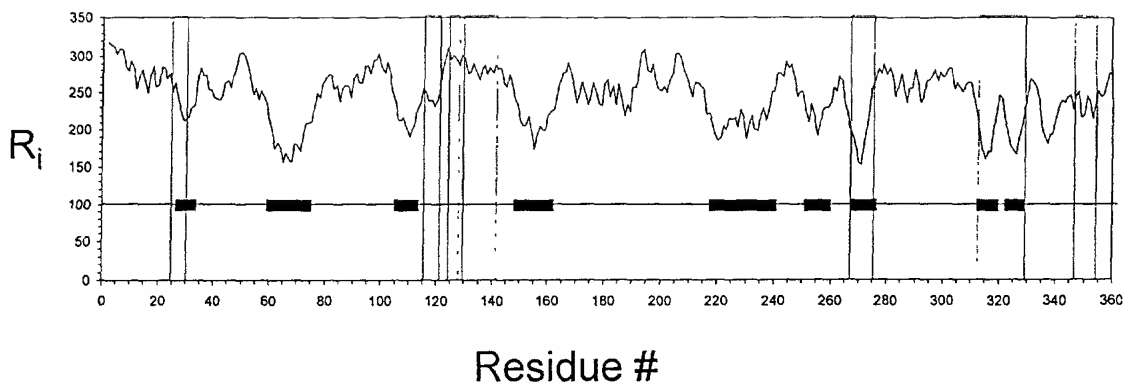
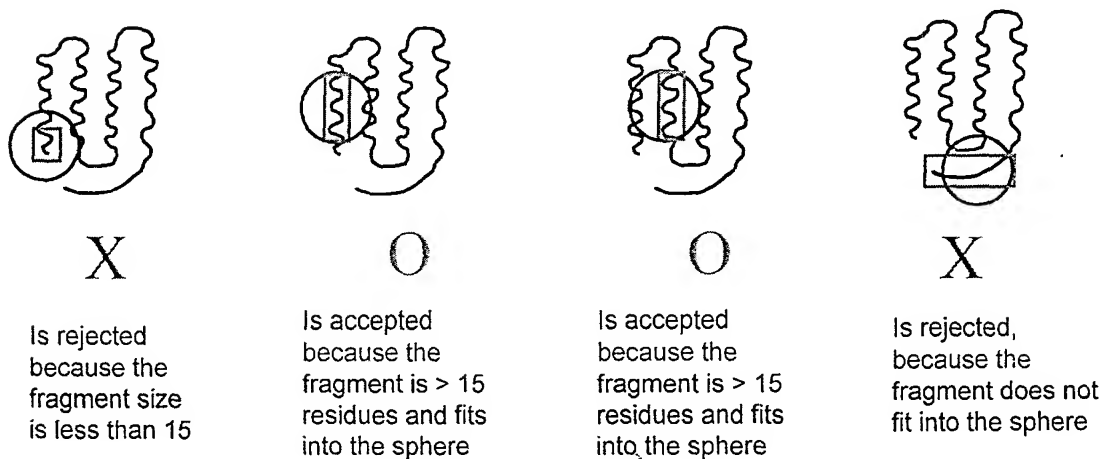


FIG. 22



(1) Pick a sphere size (21 angstroms, like Go-Gilbert) and a disruption threshold; (2) Scan protein using segments at least the average number of residues for that sphere size or greater (e.g., >15 for 21 angstrom sphere); (3) Check the disruption of all the compact fragments identified in step 2. If the fragment has a disruption above a threshold value, keep it; otherwise, throw it out; (4) If the compact unit is disruptive, increment the schema disruption measure for all of the residues in the fragment by one. This indicates that crossovers within the fragment are disfavored.

FIG. 23

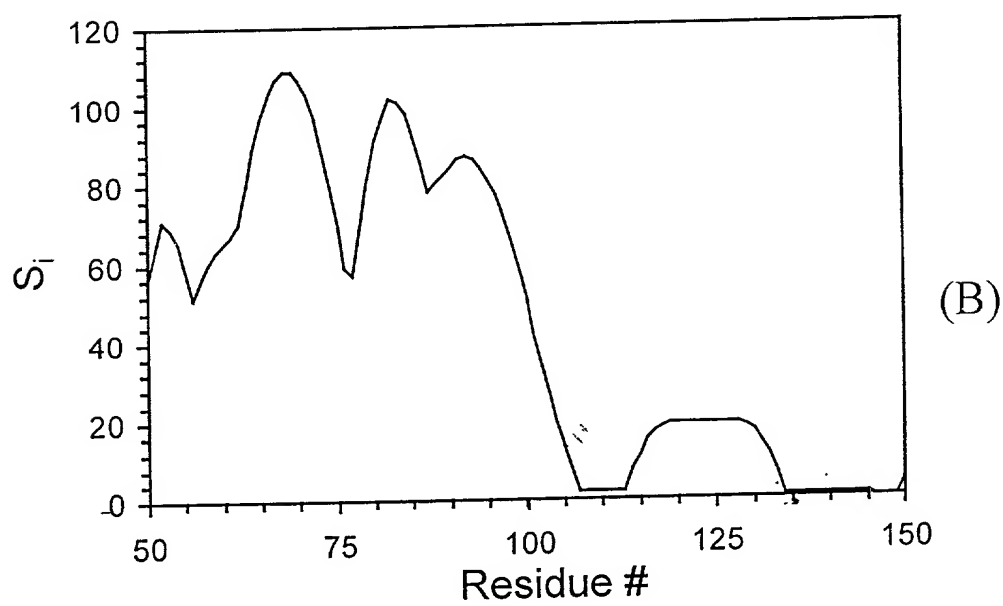
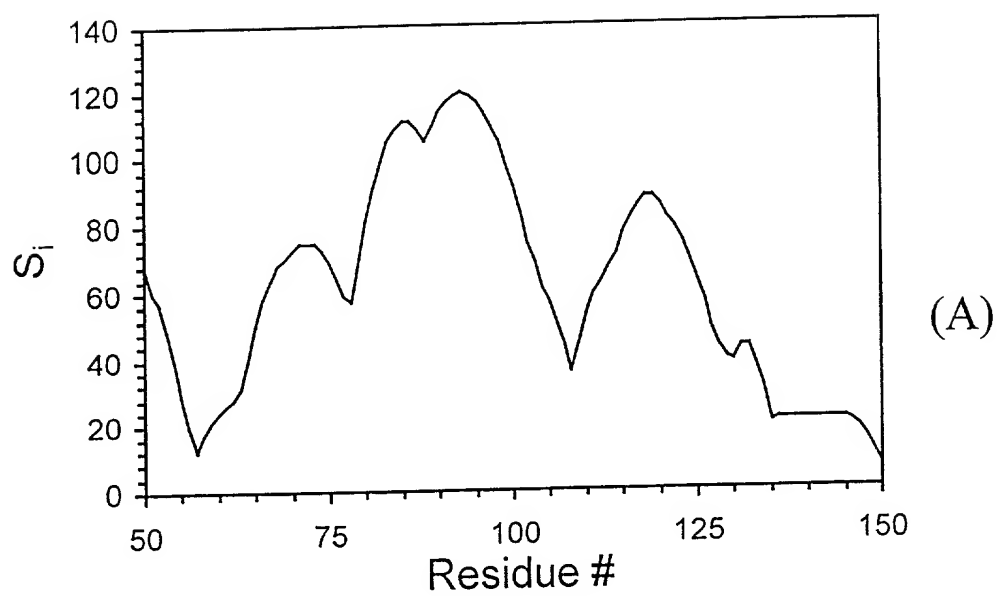


FIG. 24

10016553-10304

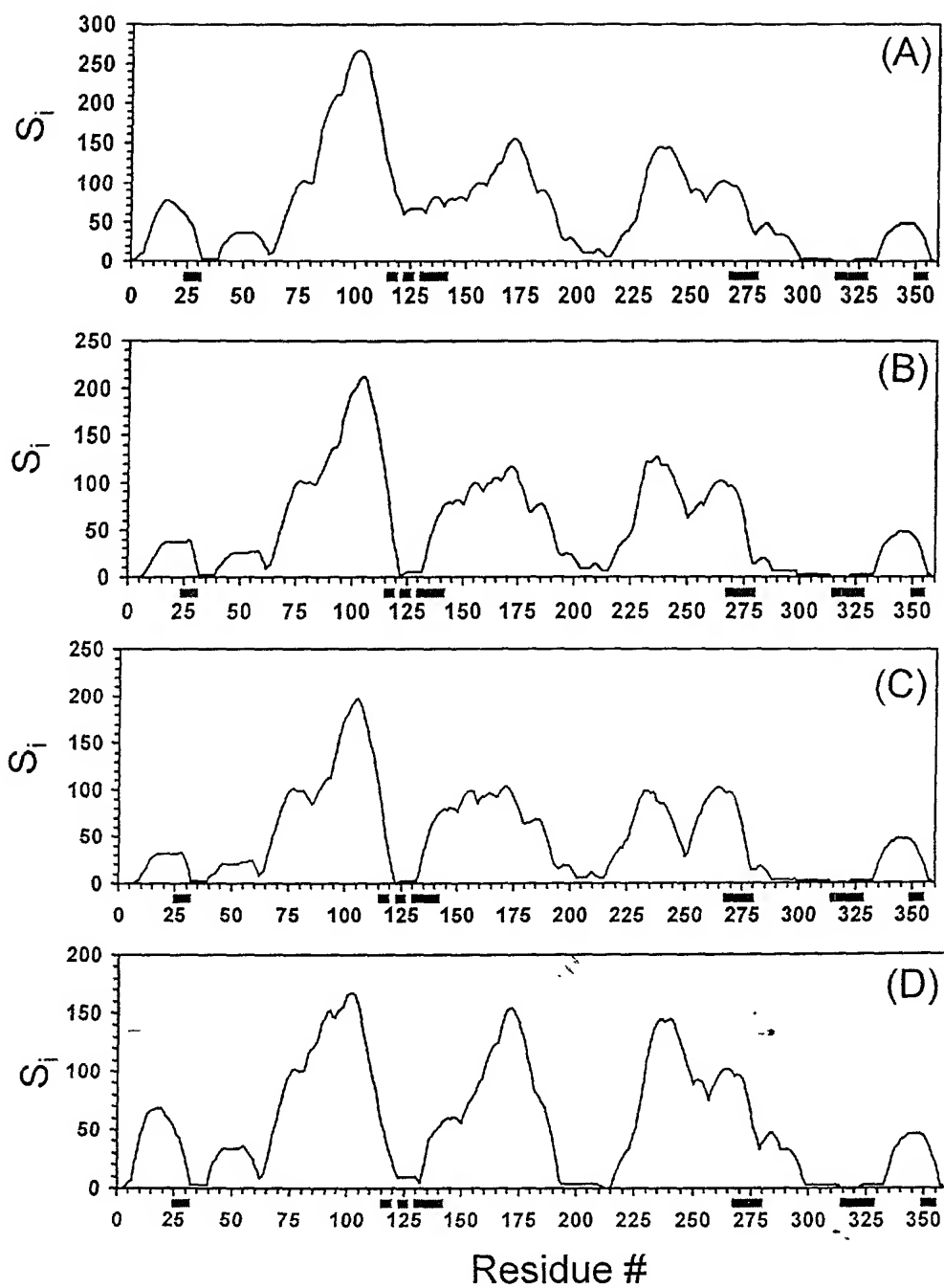


FIG. 25

1001668-103601

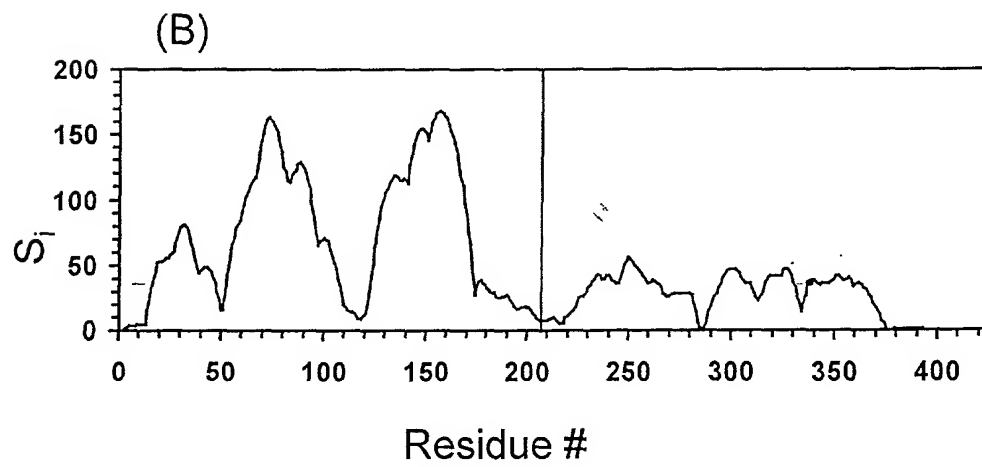
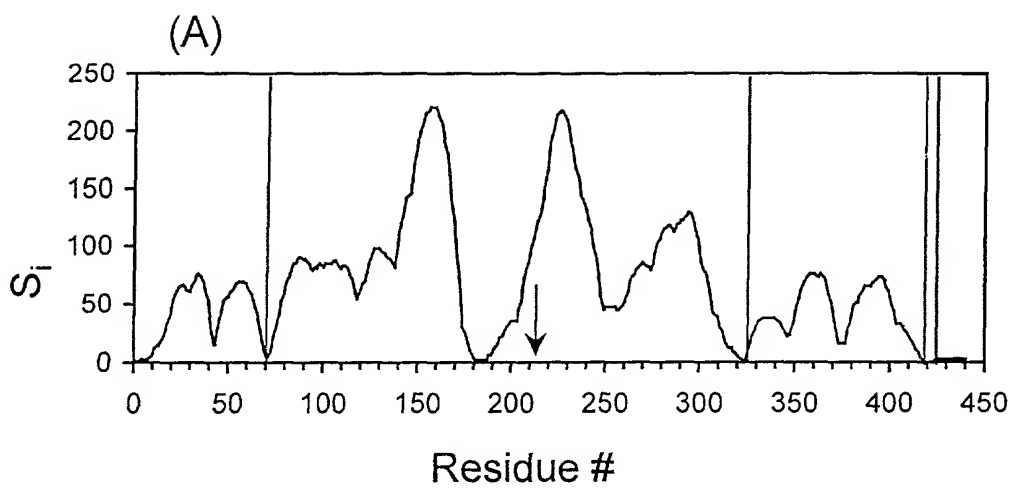


FIG. 26

FIG. 27A

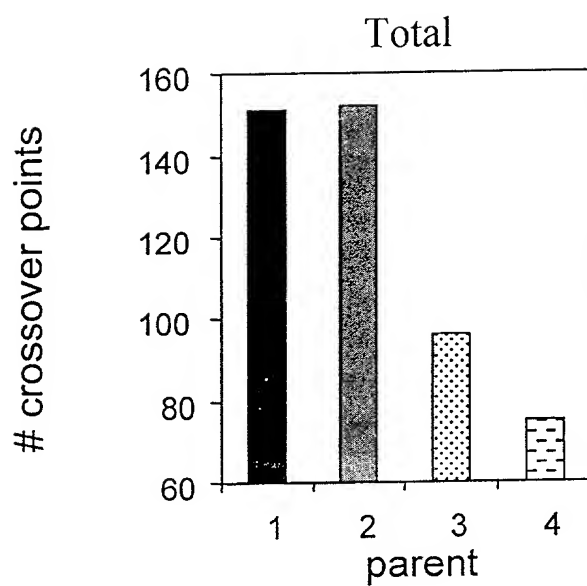


FIG. 27A

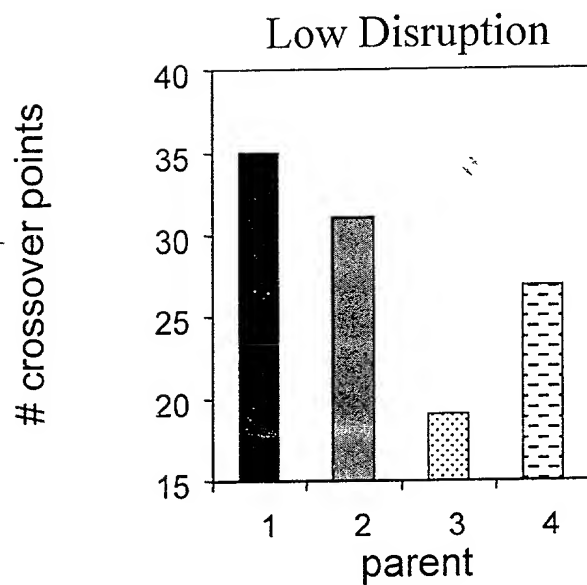


FIG. 27B

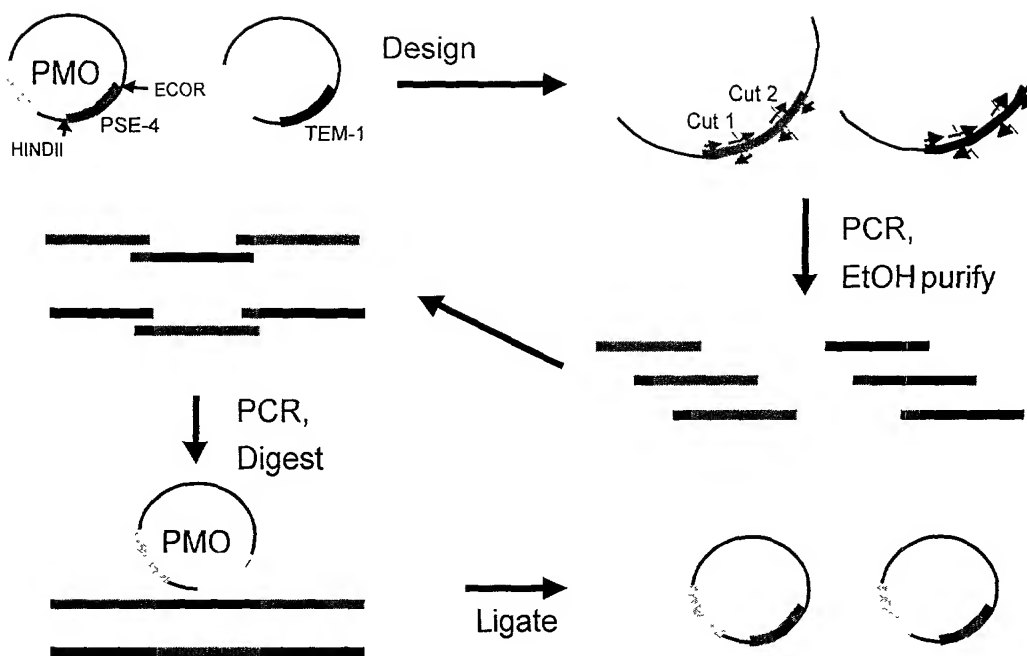


FIG. 28

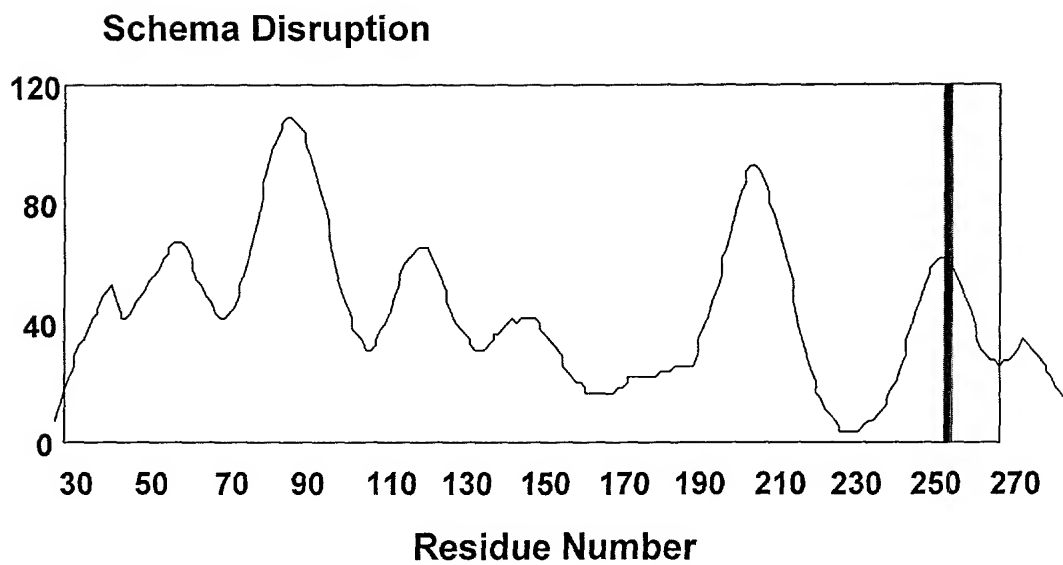


FIG. 29

Schema Disruption

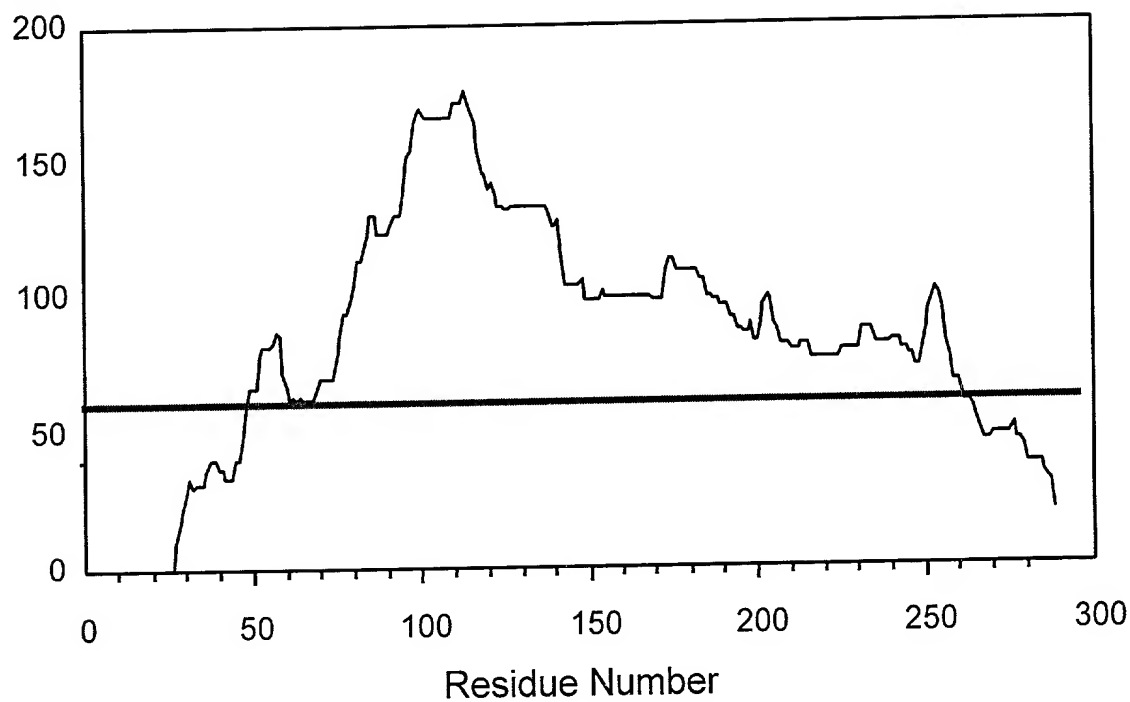


FIG. 30

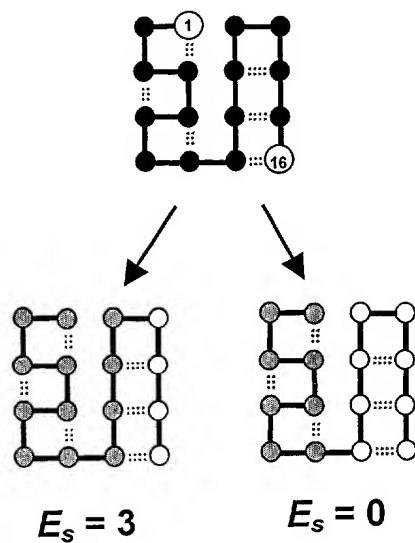


FIG. 31A

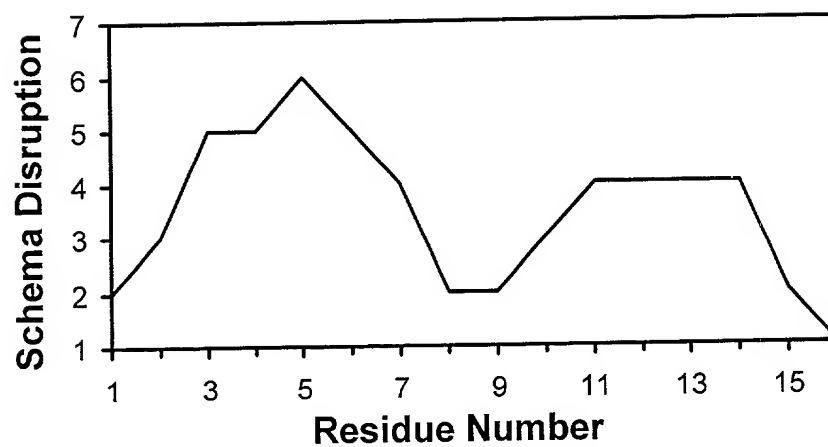


FIG. 31B

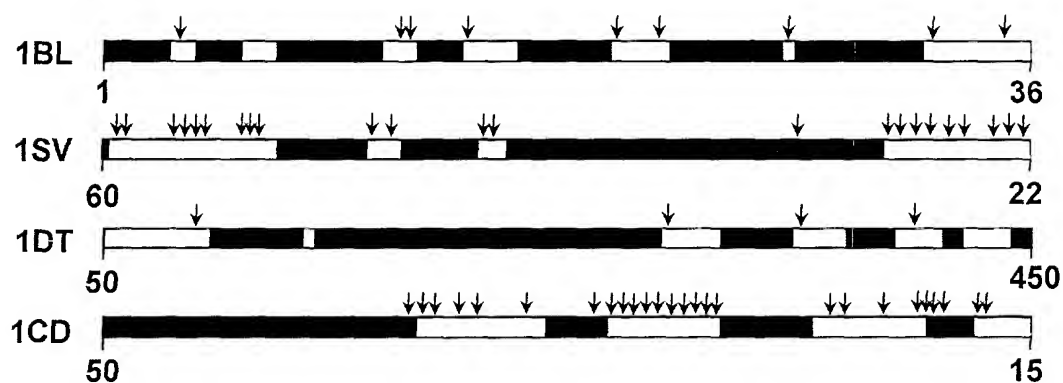


FIG. 32

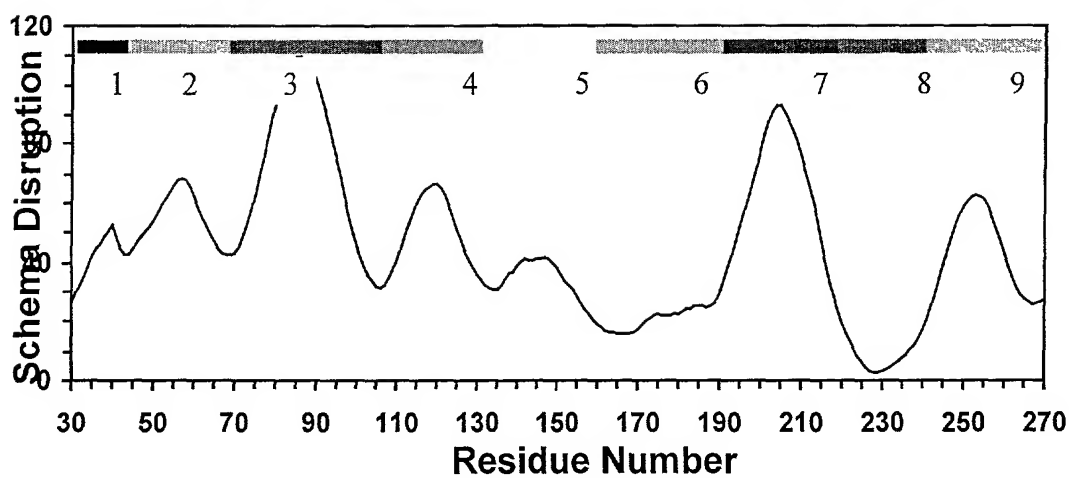


FIG. 33

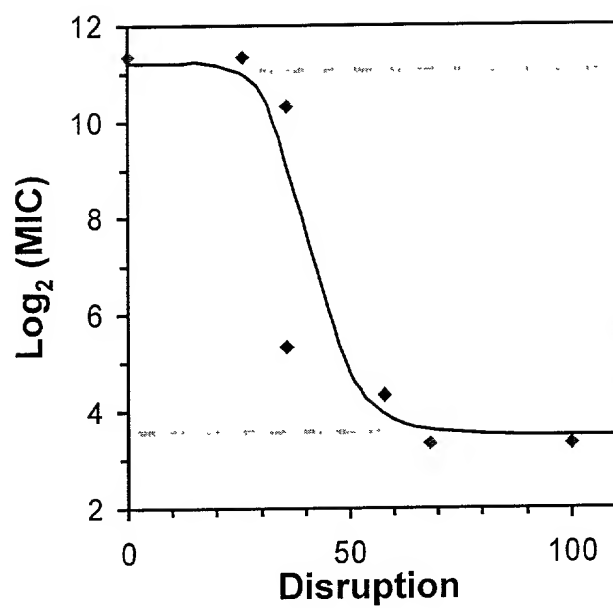


FIG. 34